

Transcriptome profile of barley aleurone differs between total and polysomal RNAs: implications for proteome modeling

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Abstract Microarray analysis of mRNA populations is routinely conducted with total RNA. However, such analyses would probably represent the translated genome (proteome) more accurately if conducted with polysomal RNA. An accurate assessment of the proteome is essential where microarray analysis is used to produce molecular markers for breeding programs. In order to determine whether significant variation occurs between these two RNA populations, the relative abundance of transcripts was analyzed in barley aleurones of intact 3.5 day old germinated seedlings, comparing total and polysomal RNAs. A total of 13,744 transcripts was detected among both populations. Of these, 714 were detected only in total RNA, and 1,541 were detected only in polysomal RNA. A surprising number of transcripts detected in both populations (6,312 gene calls or 46% of the compared transcripts) differed significantly between populations. Almost half of these (2,987) were more abundant by at least two-fold, depending on the RNA source, and expression was often biased toward specific functional classes of genes. Transcripts encoding hydrolytic enzymes for the mobilization of stored seed macromolecules were more highly represented in total RNA, rather than polysomal RNA. These

included proteinases, nucleases and carbohydrases. Genes for ribonucleoprotein complexes, nucleic acid binding and components of ribosomes were more abundant in polysomal RNA. Among genes with signal intensities of 1,000 or more, hydrolases were greatly over-represented in total RNA, whereas ubiquitin, histone and kinase related genes were mainly represented in polysomal RNA.

Keywords Aleurone · Barley seed · Microarray · Polysomal mRNA · Transcriptional profiling · Proteome

Introduction

Microarray analyses typically reflect the relative levels of thousands of transcripts in various organs and following various treatments. These analyses are often used to give a predictive assessment of the proteome and metabolome. Total RNA is normally used, even though polysomal RNA represents the translated genome. Total RNA may be unsatisfactory for several reasons. Numerous post-transcriptional controls may influence gene expression. Total RNA may contain a number of untranslated transcripts. If translated, the levels of translation products may bear no relation to apparent expression based on relative hybridization signal intensity. Ribosomes may be limiting, and each mRNA must compete for ribosome binding sites. A wide variety of subsequent

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translational and post-translational controls then determine final gene expression at the protein level. Considering this, proteome models based on polysomal RNA should be more accurate than total RNA, since ribosome binding and, presumably, translation, is reflected in the RNA source. However, polysomal RNA is not used in microarray analyses.

Transcriptome profiling with total RNA is informative for determining which genes are upregulated or downregulated. Assessment of the proteome is more important where agronomic properties, such as seed quality, are concerned. The malting quality of barley cultivars is a complex multigenic trait (Hayes et al. 2003) that is largely the product of various hydrolytic enzymes (e.g., α - and β -amylases, proteinases) and the signaling systems that control their expression, such as those related to GA and ABA hormones. Since relative levels of hydrolytic enzymes are important, polysomal RNA would seem to be the logical choice for microarray analyses to develop molecular markers for malting quality. Malting is a regimen of germination under controlled conditions designed to optimize the hydrolysis of stored macromolecules and structural components to suit specific brewing requirements. The aleurone layer surrounds the non-living cells comprising the starchy endosperm. Following germination, a burst of gene activity occurs. Hydrolytic enzymes produced in the aleurone are secreted into the starchy endosperm, providing nutrients to the germinating embryonic axis (Bamforth and Barclay 1993; Briggs 1978). Maximal activity of the most important malting enzymes, α -amylases, typically occurs 5–7 days after the onset of imbibition (dpi). This is preceded at about 4 dpi by a peak in α -amylase mRNA levels (Chandler and Jacobsen 1991; Skadsen 1993). In order to understand this process and develop molecular markers for malting quality, it is necessary to utilize the most appropriate mRNA population.

Comparisons of microarrays conducted with total RNA vs. polysomal RNA have not been reported in plants. We conducted microarray analysis of a single seedling stage to provide a snapshot of the transcriptome as represented by both transcript populations. Depending on the type of microarray study performed, significant changes in the number of expressed genes typically ranges from the tens to several thousand. What would happen to the conclusions drawn from such studies if as many, or more,

expressed genes were influenced simply by the source of the RNA? In the following study, we demonstrate that the expression differences between total RNA and polysomal RNA are at least as large as treatment differences reported in typical microarray studies to date.

Materials and methods

Plant material

Seeds of barley (*Hordeum vulgare* L., Morex cv.) were obtained from the USDA-ARS National Small Grains Collection, Aberdeen, ID. Seeds were sterilized in 1% (v/v) hypochlorite for 10 min, rinsed extensively, imbibed for 8 h, sown onto damp Kimpack cellulose wadding paper in covered plastic trays and incubated in complete darkness at 21°C for 1–4 days. For developmental RNA blot analysis, kernels were frozen in liquid nitrogen and stored at –70°C, after first removing the hulls, root/shoot axes and scutella. For microarray analysis, aleurones from 3.5-day-old seedlings were carefully removed and lightly brushed to remove surface contamination by starchy endosperm. Aleurones were divided into two subsamples (one for isolation of total RNA and the other for isolation of polysomes), frozen and stored, as above. Three biological replicates were conducted at different times.

RNA extraction

Total RNA from 1- to 4-day-old caryopses was extracted as described in Skadsen (1993) and used to determine the developmental expression profile for high-pI α -amylase. Age was counted from the initiation of imbibition (dpi). Seedlings at 3.5 dpi were used for microarray analysis. Total RNA from aleurones was extracted in the same manner, but aurointricarboxylic acid was omitted, and 5 mM ribonucleoside–vanadyl complexes (RVC; Sigma) were included as an RNase inhibitor (Berger and Birkenmeier 1979). Polysomes were extracted as per Skadsen and Scandalios (1986), except that 2.0 M sucrose pads were used in ultracentrifugation. RVC (5 mM) were also included in the homogenization buffer. The polysome pellet was rapidly dissolved in RNA extraction buffer (Skadsen 1993) without

aurintricarboxylic acid and extracted with an equal volume of phenol. Total polysomal RNA was then precipitated with 0.3 M KOAc (pH 5.5) and 2.5 volumes of ethanol. Polysomal and total RNAs were collected from aleurones in each of three biological repetitions.

RNA blot analysis

Preparation of RNA gel blots, preparation of ^{32}P -dCTP radiolabeled probe (Feinberg and Vogelstein 1983), hybridization and washing conditions, and autoradiography were conducted as previously described (Skadsen et al. 1995). Following hybridization at 62°C, blots were rinsed at 62°C and exposed to x-ray film. All RNA gels contained 8 µg total RNA per lane. Blots were probed with the high pI α -amylase clone pM/C (Rogers 1985) and a barley 18S rRNA cloned in our lab (GenBank AY552749).

cDNA synthesis and microarray hybridization

The Affymetrix Barley1 GeneChip was used to probe total RNA and polysomal RNA. The array contains 22,792 probe sets, which are mainly derived from 350,000 high-quality ESTs from 84 cDNA libraries (Close et al. 2004). The 22,792 probe sets represent about 21,439 unique barley genes. For simplicity, probe sets are referred to as genes. RNA was further purified using RNeasy cartridges (Qiagen, Valencia, CA). Ten µg of RNA was used as a template for cDNA synthesis using the One-Cycle cDNA Synthesis kit (Affymetrix). SuperScript II RT was used for first strand synthesis in a 20 µl reaction. *E. coli* DNA polymerase I was used for second strand synthesis in a 150 µl reaction. Double stranded cDNA was purified by using the GeneChip Sample Cleanup Module kit (Qiagen). Six µl was used to produce biotinylated-labeled cRNA using 3'-Amplification Reagents for IVT Labeling kit (Qiagen), containing T7 RNA polymerase. Twenty µg of cRNA was hydrolyzed to 35–200 bp with fragmentation buffer supplied with the GeneChip Sample Module kit (Qiagen). Ten µg was hybridized to each Barley1 chip. Hybridization occurred at 45°C for 16 h in an Innova incubator. Microarrays were processed on the GeneChip Fluidics Station 450 using the EukGE-WS2v4_450 fluidics protocol and scanned immediately on an Affymetrix GeneChip Scanner 3000. Hybridization and micro-

array scanning were performed by the University of Wisconsin Biotechnology Center Gene Expression Unit.

Microarray data analysis

Scanned images were analyzed using GeneChip Operating Software 1.4 (GCOS1.4; Affymetrix). Target intensity values (TGT) were set to 1,000 for all chips to make them comparable. CEL files were imported to GeneSpring GX 7.3 software (Silicon Genetics, Redwood, CA) for further analysis. GC-RMA was selected to normalize the signal intensity values within GeneSpring GX 7.3 (Silicon Genetics, Redwood, CA). GC-RMA normalizes the data using the Robust Multi-Array (RMA) expression measure taking into account GC content of the probe sequences.

The normalized data were filtered by present ($P < 0.05$), marginal ($P = 0.05$ – 0.065), or absent ($P > 0.065$) calls using Affymetrix GCOS 1.4. Present calls were also assigned if at least two repetitions were scored as marginal or better. Genes were scored as absent if at least two repetitions were called absent. The genes called absent in both total RNA and polysomal RNA populations and genes with higher intensities but called absent in one sample population were excluded from the gene list used for significance analysis by SAM (Significance Analysis of Microarrays; Tusher et al. 2001). This provided a list of 13,153 comparable genes. The median false discovery rate was 0.33%. In order to view the most salient expression differences between total RNA and polysomal populations, genes were selected using a two-fold increase or decrease in signal as the cutoff between RNA comparisons following significance analysis using SAM (Tusher et al. 2001). In order to compare expression levels between RNA populations, the mean hybridization intensity in total RNA was compared to that in polysomal RNA for each called gene. The mean of each was displayed on a scatter plot, and values were subjected to regression analysis using Sigma Plot (Systat Software Inc.).

Expression data analysis

BarleyBase and TAIR Arabidopsis database annotations were used, conducting BLASTN searches against UniProt databases using an *e* value of 10^{-20}

as the cutoff. Genes with higher *e* values were placed in the “unknown” class. Functional classification of transcripts (cellular components, biological processes and molecular functions) was done according to BarleyBase tool Expression2Function (Shen et al. 2005).

Results

Aleurones for microarray analysis were harvested at 3.5 days from the beginning of imbibition. At this time, high-*pI* α -amylase mRNA approached maximal levels, which precedes high levels of general hydrolytic enzyme activity. This was confirmed in the aleurones under study by analyzing 1- to 4-day-old seedlings by RNA blots probed for high-*pI* α -amylase mRNA (Fig. 1). RNA from 3.5-day-old aleurones was also analyzed and showed that both the total RNA and polysomal RNA were intact. RVCs were highly effective in preserving intact polysomal mRNA. Without it, considerable degradation of α -amylase and other mRNAs occurs (unpublished results). Considerably less α -amylase mRNA occurred per μ g of polysomal mRNA than occurred in total RNA (Fig. 1), corresponding to previous findings (Skadsen and Tibbot 1994).

In total, 13,744 genes were found to be expressed (called present) in the aleurone. Utilizing the 13,153 genes called present in both total RNA and polysomal RNA and a false discovery rate (FDR) of 0.33%, the expressions of 6,312 genes were found to differ significantly between total and polysomal RNA. This represented 46% of the total transcriptome detected by the Barley1 chip. By comparing transcripts in total RNA and polysomal RNA, 2,987 (22% of the

transcriptome) were found to differ significantly ($P = 0.05$) by two-fold or more in abundance between the two RNA populations. These findings imply that there is little correlation between gene expression in both populations. However, regression analysis done with the 13,153 comparable genes called present in both total RNA and polysomal RNA populations showed a strong general correlation ($R^2 = 0.86$, $P < 0.0001$; Fig. 2).

The BarleyBase Function2Expression database was used to assign genes to various cellular components, biological processes and molecular functions (Table 1). The 2,987 genes with two-fold or greater differences in expression level between total and polysomal RNAs were compared. This included 1,429 genes more highly expressed in polysomal RNA and 1,558 genes more highly expressed in total RNA. Among the cellular components, greater numbers of genes were represented in polysomal RNA for intracellular components, chromosomes, cytoplasm, and ribonucleoprotein complexes, while greater numbers of genes were represented in total RNA for membranes and proteins integral to membranes (Table 1). Major differences were found in many biological processes. This was most pronounced among the nucleic acid metabolism genes; over 5.4 times as many of these genes were more highly expressed in polysomal than in total RNA. This was also true of genes for regulation of metabolism (5.1-fold), amino acid and derivative metabolism (3.9-fold), organic acid metabolism (3.5-fold), signal transduction (3.3-fold) and biosynthesis (1.8-fold). The reverse situation was also true. Large differences were also found favoring total RNA, especially in carbohydrate metabolism (3.7-fold) and catabolism (2.3-fold). Biases were also found in molecular

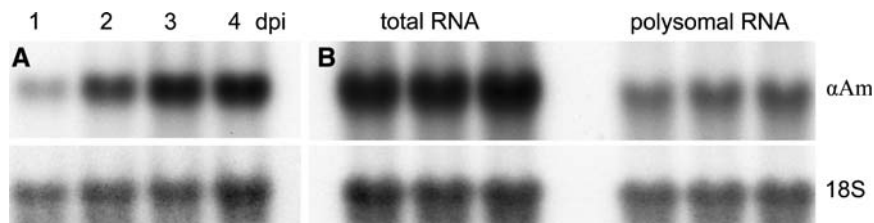


Fig. 1 RNA blot analysis showing developmental stage of seedlings with respect to α -amylase gene expression and integrity of RNAs used for microarray analysis. Panel (A)—Kernel RNA from 1, 2, 3 and 4 dpi seedlings. Panel (B)—

Three repetitions of RNA from 3.5 dpi aleurones. Total RNA (left) and polysomal RNA (right). Blot was probed with 32 P-dCTP-labeled high-*pI* α -amylase cDNA clone, stripped, and then reprobated with an 18S rRNA clone

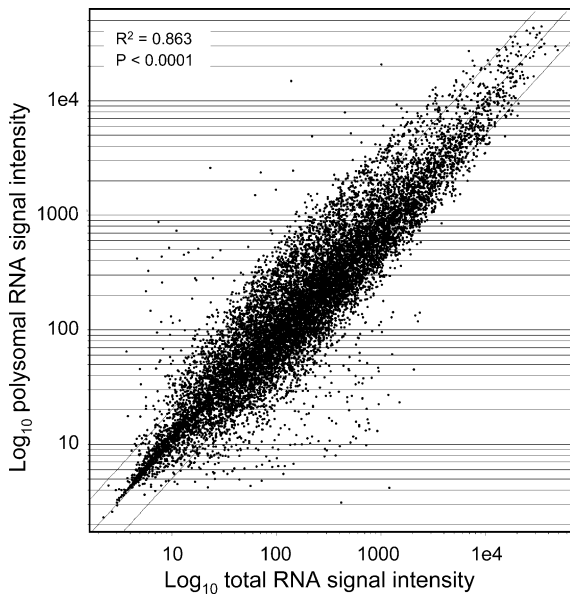


Fig. 2 Scatter plot showing correlation of gene expression detected in total RNA vs. polysomal RNA in 3.5 dpi seedling aleurones. Each point represents the \log_{10} of total vs. polysomal cRNA hybridization intensity for a single gene (average of three repetitions). Outer diagonal lines represent two-fold differences in expression between RNA sources for a particular gene. A total of 13,153 genes are represented

functions. Some of the more salient differences favoring expression in total RNA by two-fold or greater include genes for catalytic, hydrolase (especially on glycosyl bonds), peptidase, protein kinase, glycosyl transferase, nutrient reservoir, receptor and transporter activities. Molecular functions with higher expression in polysomal RNA included nucleic acid and protein binding, ribosome constituents, transcription regulator and transcription factor activities. The numbers of genes represented in total RNA only vs. polysomal RNA only also display qualitative biases in several categories (Table 1). Among biological processes, no gene categories were appreciably more highly represented in total RNA. Particularly large biases favored expression in polysomal RNA for signal transduction, cell growth and/or maintenance, amino acid and derivative metabolism, biosynthesis, nucleotide and nucleic acid metabolism, physiological processes, metabolism, protein metabolism and others. Therefore, any model of aleurone cell function is likely to be biased by the RNA source.

In addition to numbers of genes expressed, it is relevant to consider the most highly expressed genes,

as these would be expected to have a greater impact on phenotype. To analyze highly expressed genes, the 1,766 transcripts in various functional classes with relative signal intensities of 1,000 or higher were sorted manually for total RNA vs. polysomal RNA expression ratios (Fig. 3). Hydrolytic enzyme genes included mainly α -amylases, proteinases, endochitinases, α -glucosidases, xylanases and nucleases. Of 62 hydrolase genes, 54 were more highly expressed in total RNA, whereas only 8 were favored in polysomal mRNA. Thirty-four of these genes were represented by total/polysomal RNA expression ratios of 2.0- to 5.5-fold. By contrast, the 48 ubiquitin or ubiquitin-related genes were preferentially expressed in polysomal RNA; 41 had total/polysomal RNA expression ratios between 0.76 and 0.24. Similarly, 18 of the 20 kinase genes were more highly expressed in polysomal mRNA (total/polysomal ratios of 0.86–0.30). The bias is even stronger among the 21 histone and histone-related genes, where all were preferentially expressed in polysomal RNA (total/polysomal ratios of 0.77–0.11). In addition, 1,805 genes were called absent due to large variation between repetitions. A high number of these (59) were histone genes, indicating that certain genes may be highly sensitive to minor changes in growth conditions, resulting in large variations in expression (data not shown). Again, the histone genes were disproportionately more highly expressed in polysomal RNA (55 of 59).

Discussion

This study found that 46% of the transcriptome (6,312 genes) differed significantly in expression level between total and polysomal RNA, according to SAM analysis. About half of these (2,987 or 47.3%) differed by two-fold or greater. In a typical microarray study, if 1,000 or more genes are differentially regulated between treatments, this would be considered a significant treatment difference. The differences we found due to RNA source alone are as great or greater than treatment differences found in most microarray studies and should cause reconsideration of the RNA source used when conducting microarray studies.

Boddu et al. (2006) used the Barley 1 GeneChip to discover 497 genes with differential expression in

Table 1 Functional classes of genes expressed in total and polysomal RNAs

	Genes with 2-fold higher expression in		Genes expressed only in		Genes on chip
	Total RNA	Poly RNA	Total RNA	Poly RNA	
<i>Cellular components</i>					
Intracellular	124 (8.0)	332 (23.2)	49 (6.9)	325 (21.1)	1981
Chromosome	0 (0)	27(1.9)	3 (0.4)	64(4.2)	150
Cytoplasm	73 (4.7)	182 (12.7)	23 (3.2)	114 (7.4)	968
Nucleus	22 (1.4)	130 (9.1)	17 (2.4)	171 (11.1)	769
Ribonucleoprotein complex	6 (0.4)	84 (5.9)	5 (0.7)	49 (3.2)	325
Membrane	313 (20.1)	54 (3.8)	60 (8.4)	63 (4.1)	867
Integral to membrane	168 (10.8)	21 (1.5)	27 (3.8)	32 (21.5)	440
<i>Biological processes</i>					
Signal transduction	10 (0.6)	33 (2.3)	2 (0.3)	22 (1.4)	156
Cell growth and maintenance	173 (11.1)	128 (9.0)	47 (6.6)	160 (10.4)	1058
Amino acid and derivative metab. biosynthesis	8 (0.5)	31 (2.2)	4 (0.6)	23 (1.5)	201
carbohydrate metabolism	83 (5.3)	147 (10.3)	21 (2.9)	120 (7.8)	863
Catabolism	85 (5.5)	23 (1.6)	22 (3.1)	29 (1.9)	385
Nucleotide and n.a. metabolism	92 (5.9)	40 (2.8)	20 (2.8)	23 (1.5)	378
Organic acid metabolism	27 (1.7)	147 (10.3)	22 (3.1)	178 (11.6)	940
Phosphorus metabolism	10 (0.6)	35 (2.4)	1 (0.1)	25 (1.6)	219
Regulation of metabolism	50 (3.2)	18 (1.3)	26 (3.6)	31 (2.0)	478
Development	11 (0.7)	56 (3.9)	7 (1.0)	62 (4.0)	366
Physiological processes	3 (0.2)	8 (0.6)	0 (0)	11 (0.7)	68
Metabolism	620 (39.8)	584 (40.9)	208 (29.1)	571 (37.1)	4909
Protein metabolism	458 (29.4)	485 (33.9)	151 (21.1)	480 (31.1)	3978
<i>Molecular functions</i>					
Nucleic acid binding	212 (13.6)	228 (16.0)	58 (8.1)	156 (10.1)	1599
DNA binding	57 (3.7)	187 (13.1)	30 (4.2)	216 (14.0)	1201
RNA binding	24 (1.5)	104 (7.3)	19 (2.7)	158 (10.3)	702
Nucleotide binding	12 (0.8)	35 (2.4)	3 (0.4)	27 (1.8)	212
Protein binding	114 (7.3)	82 (5.7)	46 (6.4)	95 (6.2)	1033
Catalytic activity	9 (0.6)	33 (2.3)	3 (0.4)	27 (1.8)	190
Hydrolase activity	525 (33.7)	371 (26.0)	157 (22.0)	375 (24.3)	3584
Isomerase activity	240 (15.4)	88 (6.2)	54 (7.6)	91 (5.9)	1007
Hydrolase activity, glycosyl bonds	13 (0.8)	21 (1.5)	0 (0)	13 (0.8)	94
Peptidase activity	59 (3.8)	4 (0.3)	16 (2.2)	14 (0.9)	179
Protein kinase activity	78 (5.0)	32 (2.2)	15 (2.1)	12 (0.8)	270
Transferase activity	52 (3.3)	14 (1.0)	24 (3.4)	30 (1.9)	426
Glycosyl transferase activity	152 (9.8)	111 (7.8)	51 (7.1)	140 (9.1)	1170
Nutrient reservoir activity	45 (2.9)	13 (0.9)	11 (1.5)	15 (1.0)	153
Receptor activity	26 (1.7)	1 (0.1)	17 (2.4)	2 (0.1)	71
Structural constituent of ribosome	28 (2.4)	4 (0.3)	17 (2.4)	8 (0.5)	173
Transcription regulator activity	4 (0.3)	72 (5.0)	5 (0.7)	44 (2.9)	281
Transcription factor activity	4 (0.3)	27 (1.9)	4 (0.6)	29 (1.9)	181
	0 (0)	17 (1.2)	4 (0.6)	22 (1.4)	129

Table 1 continued

	Genes with 2-fold higher expression in		Genes expressed only in		Genes on chip
	Total RNA	Poly RNA	Total RNA	Poly RNA	
Transporter activity	146 (9.4)	53 (3.7)	35 (4.9)	50 (3.2)	547
Protein transporter activity	7 (0.4)	19 (1.3)	0 (0)	11 (0.7)	66

Genes to functional classes using the BarleyBase Function2Expression module. Descriptions of functional classes are in the ontology website: <http://www.geneontology.org>. Comparisons were made between the numbers and percentages (in parentheses) of genes with two-fold or higher expression differences (columns 1 and 2) and between genes detected in total RNA only or polysomal RNA only (columns 3 and 4). The number of genes in each functional category is given in column 5. Percentages of genes corresponding to a given class were derived by dividing numbers of genes by genes with two-fold higher expression in total RNA (1,558), two-fold higher expression in polysomal RNA (1,429), expression only in total RNA (714) and expression only in polysomal RNA (1,541). The numbers of genes in each class in the aleurone transcriptome were estimated by multiplying the number of genes in a class represented on the entire chip by the ratio of total genes detected (13,744) to genes represented on the chip (21,439). Nucleic acid = n.a.

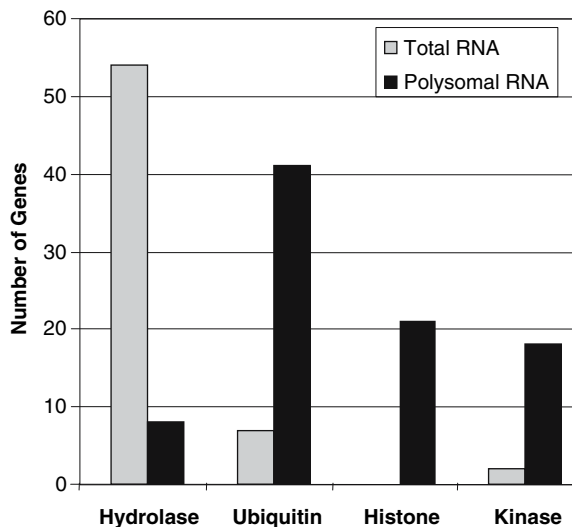


Fig. 3 Biases in expression in either total RNA or polysomal RNA for highly expressed genes in some functional classes. Numbers of genes with hybridization intensities over 1,000 (represented either as total RNA or as polysomal RNA) are represented

barley spikes infected with the fungal pathogen *Fusarium graminearum*. Svensson et al. (2006) found 2,735 with two-fold or greater differences in gene expression between total mRNAs from control and cold-treated barley plants using the Barley1 GeneChip. This represented 24.6% of the transcriptome. Chen and An (2006) used this chip to detect genes regulated by GA and ABA in isolated barley aleurones. They found 1,328 up-regulated by over three-fold in response to GA and 206 down-regulated by ABA. Under other conditions, relatively few gene

differences may be found, increasing the importance of using the proper mRNA population. Caldo et al. (2004) used the Barley1 GeneChip to examine the interaction between barley and the biotrophic powdery mildew pathogen *Blumeria graminis* f. sp. *hordei* (*Bgh*). They identified 22 host genes with divergent expression in incompatible and compatible barley–*Bgh* interactions. Bethke et al. (2005) treated isolated rice aleurones with GA and ABA. Using a rice microarray to probe RNA, fewer than 60 genes were found to be specifically up- or down-regulated by at least two-fold by either hormone. In our study, 2,987 genes were found to differ by at least two-fold in expression level simply by comparing aleurone transcripts in total vs. polysomal RNAs, which compares to the larger treatment differences found in microarray studies.

In general, transcript levels for each gene were highly correlated between total and polysomal RNA populations (Fig. 2). However, the finding of high numbers of non-correlated gene expressions suggests that the RNA source used must be selected for the study's intent. Thus, if the pattern of gene activation is important, total RNA would suffice. However, total RNA has not yet been shown to be an adequate RNA source for modeling the proteome. The correlation between expression in total RNA and the proteome has received little study. Pradet-Balade et al. (2001) reviewed studies of mRNA levels and their correlation with corresponding protein levels (in yeast and mammalian cells) and concluded that mRNA abundance is a poor indicator of protein abundance. Gygi et al. (1999) quantified 136 yeast mRNAs and their corresponding

proteins and found no correlation between the two. A recent study of correlations between transcripts in total RNA and a broad range of metabolites found in developing tomato fruit reached a similar conclusion. The general level of correlation was relatively low, and it was suggested that posttranslational mechanisms dominate metabolic regulation (Carrari et al. 2006).

Of the 13,744 total called genes in this study, 1,541 were expressed in polysomal RNA only, while 714 were expressed in total RNA only. Since all transcripts in polysomal RNA must also occur in total RNA, it is not possible to be present in the former but absent in the latter, in absolute terms. Where this occurs in the analysis, it means that there is no appreciable difference in hybridization intensity between the 11 matched and 11 mismatched 25-mer oligonucleotide probe sets. This could result for a particular gene if higher background hybridization occurred in total RNA, causing the GCOS 1.4 program to call the gene absent. This may be more prevalent in low abundance genes. In both of these populations, only a few genes had hybridization intensities over 300. This would not affect predictions of metabolic functions if there were no bias as to the types of genes represented. However, significant bias occurred (Table 1).

Since malting quality relates primarily to the activities of numerous hydrolytic enzymes, microarray analysis for the purpose of discovering molecular markers should relate to the proteome. The time point of 3.5 days from the initiation of imbibition is an important stage. Levels of high-*pI* α -amylase mRNAs are close to their developmental maxima, and α -amylase enzyme activity begins to increase above background levels as repressive ABA hormone levels decline (Skadsen 1993; Skadsen and Tibbot 1994). Since polysomal RNA would be expected to represent the proteome more closely than would total RNA, it would be expected that highly expressed genes such as α -amylases would be highly represented in polysomal RNA. Microarray probing of the transcriptome found the contrary result. This is in line with our previous findings with RNA blots and with the RNA blot results of Fig. 1. Both high- and low-*pI* α -amylase mRNAs are much more highly represented in total RNA throughout germination and early seedling development, both on a per μ g RNA basis and on a per g fresh weight basis (Skadsen and Tibbot 1994). Less than 4% of the α -amylase mRNAs ever occur on polysomes at any time. The correct interpretation is not known because

quantitative proteomics in comparison to mRNA levels has not been studied in malting barley. These results suggest that caution is required before accepting any model, since a wide variety of post-transcriptional controls will ultimately determine the expressions of many genes. The use of polysomal RNA in transcriptome analysis could provide new insights into complex patterns of gene expression, including those that control seed quality traits.

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